EXHIBIT 10

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IHR ZEICHEN / YOUR REF	UNSER ZEICHEN / OUR REF	DATUM / DATE
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Re: European Patent No.: 0308378
(EP Appln. No.: 88830365.8-2105)
Proprietor: YEDA RESEARCH AND DEVELOPMENT CO., LTD.
Opponent: AMGEN INC.

In preparation of Oral Proceedings, Opponent II respectfully submits the following:

<u>Introductory Remarks:</u>

1. Throughout this letter, the designation of documents as chosen in the consolidated list submitted by the proprietor will be used. Newly cited documents will be designated **DA (No)**. A list of newly cited documents is attached to this letter **(Annex 1)**.

2. In this letter, the following terms shall have the meaning indicated below:

TNF-receptor: A TNF binding protein as integrated in a cell membrane, i.e. comprising an extracellular portion, a transmembrane region and a cytoplasmic portion;

TNF-inhibitor: A protein corresponding to the extracellular portion of a TNF-receptor.

Urine-derived TNF-inhibitors:

TNF-bp I: The urine-derived TNF-inhibitor which was later determined to be a TNF-binding protein and designated TBP-1 in **D20**.

TNF-bp II: The TNF-inhibitor called TBP-II in **D20**.

With respect to TNF-bp I, we will distinguish between "full length" inhibitor (30 kD) and "truncated" form thereof (26 to 28 kD) disclosed in the opposed patent.

Recombinant TNF-Inhibitors

sTNF-RI: a recombinant, soluble form of the high affinity p55 TNF-receptor type I.

sTNF-RII: a recombinant soluble form of the low affinity p75 TNF-receptor type II.

3. **Annex 2** attached to this letter is a table of contents reciting the headlines of the respective section or providing keywords, where appropriate.

1. Priority

It is noted that the Proprietor does not deny that claims 3 to 8, 11 to 26 and 31 to 33 are not entitled to priority from IL83878 nor does he object to the statement that claims 9 and 10 and 27 to 30, as far as not being dependent on claims 1 and 2, also do not enjoy the priority of the Israeli priority document.

2. Extension beyond the content of the application as filed (Art. 123(2) EPC)

The Proprietor contends that the subject matter of claim 11 does not contravene Article 123(2) EPC.

Allegedly the molecular weight of the claimed protein is not an essential feature, (see Item 4.2 of letter dated August 22, 1996) and thus may be removed from the claim. This conclusion is in error.

2.1 The molecular weight of a protein is an essential feature per se, even in cases where this is not reflected by the specification. Support for this view is to be found in every encyclopedia, where the molecular weight is always one of the first mentioned characteristics of a protein.

2.2 In the present case, the importance of the molecular weight is also apparent from the patent specification, since the one and only protein preparation disclosed in the specification is always characterized by its molecular weight observed following chromatography on ultrogel AcA44 or following SDS gel electrophoresis. The specification does not disclose any other protein having TNF inhibitor activity, it does not indicate that such proteins exist and it does not identify or characterise TNF inhibitors other than that of claim 4. The patent also does not disclose any process for obtaining a TNF inhibitor other than that of claim 4. It should be noted that the process of claim 11 lacking the indication of a certain molecular weight was not part of the specification as originally filed. Rather, the B1-specification has been amended in order to comply with extended claim 11.

It is submitted that deleting the molecular weight from claim 11 constitutes the inadmissible attempt to extend the process to other TNF inhibitor proteins, for example, TNF inhibitor proteins having a higher molecular weight.

2.3 As will be discussed below (Section 3.1.1) the opposed patent does not even enable a full length TNF-bp I. The patent provides disclosure relating to a 26 to 28 kD protein only, rather than providing any disclosure relating to a full length, i.e., 30 kD protein. This fact further demonstrates that it is absolutely unjustified on the basis of the original disclosure to extend the claim to a process allowing the production of TNF inhibitors other than those actually disclosed.

3. Insufficiency of disclosure (Article 83 EPC)

3.1 Lack of sufficient disclosure of claims 1 to 3 and claims dependent thereon

3.1.1 The opposed patent does not disclose the isolation of a full length TNF inhibitor. Rather, the protein disclosed in the opposed patent represents a truncated form obtained by degradation during the disclosed process of purification. This is supported by **D20**, cited as an expert's opinion, in the paragraph bridging pages 1534 and 1535, stating:

> "When isolated by a multistep chromatographic procedure, the TNF binding protein turned out to be some what smaller than after ligand affinity purification (27,000 (10) compared to about 30,000).... A likely explanation for the difference in molecular size is the higher probability for proteolytic degradation in the more lēngthy manipulations involved in the chromatographic purification as compared to affinity purification.

Reference (10) cited in the above provided quotation from **D20** is a publication by Engelmann et al., J. Biol.Chem. 264, 11974-11980, 1989, copies of which are enclosed herewith (**DA24**). According to page 11975, right column, **DA24** applied the very same purification procedure as taught by the opposed patent. Applying the same procedure necessarily results in obtaining the same product, which in **DA24** is indicated to be in a protein having a molecular weight of 27,000 D as compared to 26,000 to 28,000 D according to the opposed patent. Since 27 kD is within the range of 26 to 28 kD, this confirms the identity of the products.

For convenience the reader, the following table juxtaposes the teaching provided in the patent and in **DA24** with respect to purification:

Process Step	Disclosure of Patent	Disclosure of **DA24**
Ultra filtration	Page 7, line 52	Page 11975, right column, "Concentration of the crude urinary proteins (CUP)"
Cut off 10 kD	Page 7, line 54	Page 11975, right column, "Concentration of the crude urinary proteins (CUP)"
CM-Sepharose	Page 7, line 56 to page 8, line 9	Page 11975, right column, "Chromatography on CM-Sepharose"
Cation exchange HPLC Mono S	Page 8, lines 10 to 22	Page 11975, right column, "Cation exchange HPLC"
Anion exchange HPLC Mono Q	Page 8, lines 23 to 33	Page 11975, right column, "Anion exchange HPLC"
RP-HPLC	Page 8, lines 34 to 35	Page 11976, left column, "Reversed phase HPLC"
SDS-PAGE	Page 8, line 47 to page 9, line 2	Page 11976, left column, "SDS-Polyacrylamide gel electrophoresis"
Micro sequencing	Page 9, lines 3 to 12	Page 11976, left column, "N-terminal sequence analysis"

Consequently, the analysis of **DA24** inevitably leads to the conclusion that the protein according to **DA24** and that of the opposed patent <u>are the same</u>. Thus, according to the admission of some of the inventors the relatively low molecular size as disclosed in the opposed patent is a consequence of proteolytic degradation due to the chosen purification method. Thus, the protein provided by the patent is not a full length TNF-inhibitor, but rather a truncated form thereof.

There is no teaching in the patent, which would provide full length inhibitor. Therefore, the proprietor is not entitled to a claim embracing a protein other than that in fact obtained according to his method, i.e., the protein having an MW on SDS-PAGE of 26 to 28 kD.

3.1.2 The opposed patent in fact does not contain any bit of a teaching concerning a TNF inhibitor other than that having the characteristics of claims 1 and 4. In compliance with recent case law of the Technical Board of Appeal 3.3.4, the patent ought to be restricted to what is actually disclosed. In this connection it is referred to T694/92 (not yet published): in the patent underlying said decision, claim 1 is directed to a method for generically modifying a plant cell, which method comprised inserting a plant promoter and a plant structural gene into T-DNA, and transforming the thus obtained T-DNA/plant gene construct into a plant cell. Further claims relate to a plant cell obtained by the claimed method as well as a plant or plant tissue grown from said plant cells. The examples supporting the claims demonstrate the expression of phaseolin in plant cells, wherein the phaseolin is under control of its own promoter. The patent description further indicates without providing any examples that the promoter and structural gene could be derived from the same or different plant sources, that a plant gene could be placed downstream either from its own promoter or from a different plant promoter and that the promoter and coding regions may also include modifications, either naturally or artificially induced and may include chemically synthesized segments. However, there are no examples other than those mentioned above.

The then competent Board held

> "... the question is whether the skilled person, on the basis of the description of the patent in suit (...) and of the prior art, would have been in a position at the priority date to carry out the method for the whole range of applications claimed without finding himself/herself in a situation were, despite using reasonable effort to make the method work, he or she would have achieved technical effect for some applications or would have achieved it only with undue burden."

In that case the Board held the claims to be unduly broad:

> "... the Board has decided that the experimental evidence and technical details in the description of the patent in suit are not sufficient for the skilled person to reliably achieve without undue burden the technical fact of expression in <u>any</u> plants cell of <u>any</u> plant structural gene under the control of <u>any</u> plant promoter and that, consequently, they do not provide sufficient support for a claim, such as present claim 1 broadly directed to such a method. "

The Board finally accepted claims to the method for generically modifying a dicotyledonous plant cell, comprising the step of inserting a plant gene comprising a <u>phaseolin</u> promoter <u>and</u> a <u>phaseolin</u> structural gene into T-DNA. Thus, the Board restricted the claims to plant a promoter and plant structural gene actually disclosed in the patent and allowed a generalization only with respect to the plants subjected to the method.

In the present case, claim 1 is broadly directed to a TNF-inhibitor having the following features:

(a) it inhibits the binding of TNF to its receptors and the cytotoxic effect of TNF;
(b) when crude urine preparations thereof are chromatographed on an Ultrogel AcA 44 filtration column, the major peak of TNF inhibitory activity elutes slightly before the majority of the protein and shows an apparent molecular weight of about 40-80 kDa; and
(c) when crude urine preparations thereof are analysed, the isoelectric point of the active protein is between pH6 and 8

or a salt, functional derivative or active fraction thereof, said active fraction having the ability to inhibit the binding of TNF to is receptors and the cytotoxic effect of TNF.

This claim encompasses, in addition to the actually disclosed embodiment of truncated TNF-bp I, full length TNF-bp I, TNF-bp II and also sFas. In view of a disclosure which is limited to proteins having a certain size, this claim must be

considered as overly broad. Moreover, in contrast to the case underlying the above mentioned decision, the opposed patent not only does not provide any examples of TNF inhibitors other than that of claim 4, it does not even provide formal support for proteins other than those having the characteristics of claims 1 and claim 4.

The Proprietor argues that there are other proteins in fractions 21 to 23 of the RP-HPLC eluate (Figure 6). Although Figure 6 in fact shows some additional bands, there is no disclosure indicating that these bands should have TNF inhibitor activity. There is also no disclosure relating to other alternative methods of obtaining higher or lower molecular weight TNF inhibitors. Therefore, in fact claims 1 to 3 and further dependent claims to the extent they do not depend on claim 4, are insufficiently disclosed.

3.1.3 In addition, there is no disclosure whatsoever relating to a functional derivative or an active fragment.

3.1.3.1 The patent does not indicate at all how a functional derivative or an active fragment should look like. The specification fails to clearly define the term "functional derivative" or identify "a precursor" or disclose what constitutes "an active fraction". Furthermore, the patent does not disclose where the active center of the molecule is, nor, which additional parts of the molecule are required for activity. There is absolutely no teaching instructing a skilled person how active fragments or functional derivatives should be designed.

3.1.3.2 Furthermore, there is no technical teaching as to the concrete steps which should be taken to fractionate the proteins. As set forth in Section 3.3.2.3.1, below, it was not possible to obtain the complete sequence of TNF-bp I. Consequently, it would not have been possible to systematically make and test fragments of the disclosed TNF-bp I.

3.2 Lack of sufficient disclosure of claims 3 to 10, claims 11 to 16 and dependent claims referring to any of these claims

Claim 3 is directed to a TNF inhibitory protein in substantial purified form, claims 11 to 15 refer to a process for isolating a substantial purified inhibitory protein, and claim 16 is directed to the protein obtained according to any of claims 11 to 15.

3.2.1 It is respectfully submitted that in view of the Proprietor's own submissions, none of the claims mentioned before is sufficiently disclosed. In the prosecution of EPa 90124133.2-2105 (EP 433900), the proprietor clearly admits that reduction to practise of the recombinant embodiments involves an inventive step. For background, EP 433900 claims priority from IL92697 **(DA25)** (filing date: December 13, 1989) and IL95064 (filing date July 12, 1990). The European patent exemplifies the cloning of cDNA and expression in CHO cells and *E. coli* cells. In the prosecution of that case, the proprietor faced an inventive step objection in view of **DA26** (Olsson et al., Eur. J. Hematol. 42:270-275, 1989), which discloses the first 20 N-terminal amino acids (wherein residues 4 and 18 are designated X and residue 14, which is "X" in the opposed patent, is incorrectly designated V). In his letter dated March 16, 1994, the proprietor submitted (**DA54**):

> "The "gap" between D1 *(i.e., **DA26**; comment added)* and the claimed subject-matter can be seen by the provision of the tools and means for expression of TBP-I in a recombinant fashion in the host cell. To fill in this gap, <u>it certainly requires inventive step</u>."
> *(emphasis added)*

DA26 does provide more information than the opposed patent. Therefore, the "gap" between the teaching of the patent and the claimed recombinant embodiment is even larger. Furthermore, according to European case law the claimed subject-matter must be reproducible without undue burden of experimentation and without needing inventive skill. Therefore, the proprietor himself inherently admitted the teaching of the opposed patent to be insufficiently disclosed.

3.2.2 Furthermore, admitted by the Proprietor in section 4.2 of his letter dated August 22, 1996, the method according to the opposed patent fails to provide a pure protein. Rather, the method provides protein preparations containing other proteins in addition to the claimed protein. In Section 4.2, the proprietor admits:

> "...Fig. 6... clearly reveals that not only a TNF-inhibitory protein in the range of 26-28 kD can be isolated but with respect to fractions 21-23 (lanes E, F and G) also TNF-inhibitory proteins having a higher molecular weight".

The above admission is supported by Figure 6 of the patent which, according to page 3, line 57, of the patent, shows samples of the active material of each step of purification. Accordingly, lanes E, F to G of the SDS gel show the protein pattern of samples obtained according to the method disclosed on page 7, line 28 to page 8, line 45 of the opposed patent, i.e. a method comprising three ion exchange-chromatography steps (CM-sepharose, mono S FPLC and mono Q FPLC) and RP-HPLC. From Figure 6 it is immediately apparent that said method does not allow to provide a purified protein, but rather a mixture of proteins of unknown identity. Neither the claims nor the patent specification as a whole provides any steps allowing to separate TNF-bp I from the contaminants visible in Figure 6.

3.2.3 The finding that the subject-matter provided by the opposed patent can not be considered "substantially purified", as commonly understood in the field is further supported by the specification of the patent, page 9, lines 10 to 11 under the heading "Automated protein micro-sequence analysis":

> "The initial yield was over 40%, indicating that the major protein in the preparation (the 27 kDa band) is related to the resulting sequence."

Two observations are noteworthy:

3.2.3.1 The initial yield is as low as 40%. It is submitted that the first residue yield of a clean sample is expected to be 70% rather than "over 40%" [LeGendre and Mastudairo, Biotechniques 6:154-159, 1988 **(DA27)** and Dixit et al., J. Biol.

Chem. 259, 10100-10105, 1984 **(DA28)**.] Thus, these data provide evidence that the sample subjected to micro sequencing still was highly contaminated.

3.2.3.2 According to line 11 of that page, the <u>major protein</u> is related to the resulting sequence. Thus, there are other (minor) proteins, accounting for up to 60% of the toal protein content. It is apparent, that in view of these data the term "substantially pure" appears to mean "comprising more than 50% impurity".

3.2.4 In fact, there are several proteins known which may co-purify with TNF-bp I from human urine. The table reproduced below indicates some of the proteins known to occur in human urine and having a pI and MW similar to TNF-bp I:

Protein	MW	pI
CD27	28,000-30,000 **DA29**	pI:7.87 **DA30**
sTNF-RII #	27-40,000 **D20**	pI:7.47 **DA30**
sFas #	30,000 **DA30**	pI:7.55 **DA30**

Additionally, Opponent II has run TNF-bp I through C8 RP-HPLC and it elutes at about 37.9% acctonitrile. When *E. coli*-derived sFas was run through C8 RP-HPLC it eluted at 38.3%. This indicates that TNF-bp I and sFas when derived from the urine will comigrate in the RP-HPLC.

Further, according to **D20**, page 1534, left column, TNF-bp I and TNF-bp II elute adjacent to each other:

> "Such contamination is not unexpected, since in the final purification step of the TNF binding proteins the two proteins elute from reversed phase HPLC column adjacent to each other (see below)".

[It is noted that the opposed patent and **DA24** indicate that TNF-bp I is eluted at 27% acetonitrile, whereas the number given above is 38.3%. However, this is not a contradiction. Due to possible differences in the type of HPLC used, the gradient used and reagent concentrations, the same molecule migrates

differently. The difference in comparative elution profiles between that disclosed above and that disclosed by **DA24** is within expected variation.]

The proteins marked by # also inhibit TNF-α, thus rendering them indistinguishable in the assays of the patent. In addition, they are co-expressed with TNF-bp I [see **DA31** (Spinas et al., J. Clin Invest. 90:533, 1992) and **DA32** (Kalinkovich et al., J. Interferon and Cytokine Res. 15: 749-757, 1995) for TNF-bp II and **DA33** (Colagiovanni et al., J. Cytokine 9: 1913, Abst. 94, 1997) for sFas].

3.2.5 With respect to the Proprietor's allegation that the larger bands are different forms of TNF-bp, it is submitted that there is no indication whatsoever that this is the case. It is known in the meantime that TNF-bp II shares some homology with TNF-bp I; however, this homology does not exceed that shared with CD40 (see e.g. **D19**, Figure 2). Since the patent does not disclose any means for distinguishing between these proteins and separating them, there is no reason to assume that TNF-bp II and only TNF-bp II should copurify with TNF-bp I.

3.3 Lack of sufficient disclosure of claims 17 to 26

The Proprietor's submissions are not suitable to demonstrate enablement for the claimed recombinant embodiment. In the following, comments on the Proprietor's allegations shall be provided.

3.3.1 <u>Availability of a cell line as source for mRNA encoding TNF inhibitory proteins (Section 5.3.2.1 of Proprietor's letter)</u>

The proprietor submits that

> "At the priority date it was well known that a great number of cells possess receptors for TNF. Thus, the person skilled in the art could have taken any cell line available to him and would have been successful."

However, this allegation is not suitable to demonstrate availability of a cell source for <u>soluble TNF inhibitor</u> mRNA.

3.3.1.1 Nowadays, it is known the TNF-inhibitors constitute the soluble extracellular domain of TNF receptors, with the TNF receptor comprising in addition to the extracellular domain a transmembrane domain and a cytosolic part. However, this relationship between TNF receptor and TNF inhibitor was not known until about 1990. In 1989, the origin of TNF-bp I was still unknown. See Wallach et al., Lymphokine Research 8: 359-363, 1989 **(DA34)**:

> "The cellular source of this urine-derived TNF-binding protein remains to be elucidated." *(page 361, last paragraph).*

This lack of knowledge is further supported e.g. by **D19**, submitted by the Proprietor. **D19**, which was published in 1990, presents data suggesting that the soluble form of the TNF receptor is structurally identical to the extracellular cytokine binding domain thereof (see abstract of **D19**, last sentence). Thus, in 1990 it still was an exciting news justifying a publication in the EMBO Journal that there may be a relation between receptor and inhibitor. Clearly this was not known at the relevant date for claims 17 to 26, i.e. the European filing date in September 1988. Consequently, at the relevant date there was no cell line known to be a suitable source for an mRNA encoding TNF inhibitors.

3.3.1.2 The above conclusion is also supported by the failure of others to identify a suitable cell line. The proprietor was not the only institution active in the research area of TNF-inhibitors. In contrast, the field was highly competitive, and competent research teams at well known companies like Boehringer Ingelheim, Hoffmann La-Roche, Genentech, and last but not least, Synergen were active in the same field. From one of these research teams, i.e., the Boehringer Ingelheim team, we know that using a mixture of oligonucleotides containing one oligonucleotide having an exactly matching sequence they were not successful in screening commercially available cDNA libraries. The sequences of these oligonucleotides are given below:

↓

EBI-1639: CA(AG)GGTAA(AG)TA(TC)AT(TCA)CA(TC)CC
EBI-1640: CA(AG)GGCAA(AG)TA(TC)AT(TCA)CA(TC)CC
EBI-1641: CA(AG)GGAAA(AG)TA(TC)AT(TCA)CA(TC)CC
EBI-1642: CA(AG)GGGAA(AG)TA(TC)AT(TCA)CA(TC)CC

The above sequences correspond to amino acids Gln^5 to Pro^{12} of the N-terminal sequence disclosed in the opposed patent. Oligonucleotide EBI-1641 contains a sequence matching exactly the nucleic acid sequence of **D19**. The oligonucleotide mixture was used to screen cDNA libraries for fibroblasts (Hs913T), and monocytes (U937) and a company-made human placenta cDNA library. No positive clone could be identified and reconfirmed in either of the aforementioned libraries using the above oligonucleotides.

In addition, in a separate experiment EBI-1642, which contains one mismatch in the sixth position (see arrow), was used to screen a commercially available HeLa cDNA library. Note that HeLa is one of the cell lines relied on by the patentee in his letter of August 22, 1996 (see Section 5.3.2.1). However, also this approach was unsuccessful.

Along the same lines, the Roche group reported in Loetscher et al., Cell 61:351-359, 1990 **(DA35)** that

> "In preliminary experiments, the more conventional cloning approach using relatively short, fully degenerate or longer best-guess oligonucleotides as probes to screen cDNA libraries had proven technically difficult."

Presumably, this group initially attempted to use the conventional cloning approach to screen certain cells (HL60, U937, Hep2 and Ag1523 cells) which had been confirmed to be TNF receptor positive (**DA35** pages 354-355). Thus, this group also experienced major difficulties, which apparently required more than routine experimentation to overcome.

Moreover, Opponents note that the technique of using longer best guess oligonucleotides is the subject of **DA36** (Lathe, J. Mol. Biol. 183:1-12,1985) which is one of the references cited in the opposed patent as a suitable approach to cloning the DNA.

3.3.1.3 In addition, the proprietor himself appears to have been innocent even in 1990 as to which library would be suitable. It is requested to carefully consider **D19**, in

particular page 3270, indicating that even in 1990, in any case sometime after the European filing date, the proprietor had some difficulties in order to determine a suitable cDNA library. See page 3270, left column, indicating that several cDNA libraries were screened. Apparently, two years later than the European filing date there was neither information available as to which library should contain a TNF inhibitor gene, nor was the approach of identifying promising cell lines using monoclonal antibodies as discussed in Section 5.3.2.1 of the proprietor's letter successful. **D19** provides the clear information that several attempts to clone the gene from libraries selected by the proprietor of the opposed patent failed.

In fact, in another patent application on which Drs. Aderka, Engelmann and Wallach are listed as inventors (**DA25**, filed December 13, 1989 cited as an expert's opinion), the proprietor admits to a failure to obtain any clones when screening cDNA libraries from HeLa cells, placental cells and liver cells. HeLa cells are identified in **D20** as expressing a cell surface protein which is cross-reactive with antibodies against affinity purified TNF-bp I. However, when screening a λgt11 cDNA library from HeLa cells no clones were identified. Similarly, no clones were identified in the liver library. Moreover, the proprietor describes the hybridization of the probe with cDNA from clones 17, 19, 131, 133 and 152 (leading to the assumption that at least 152 clones were initially identified) in the placental library. After purifying and reprobing identified clones, only 3 clones hybridized to all three of the probes; and when sequenced these clones were found to be false positives!

These facts on their own should lead to the conclusion, that it would be an undue burden for a skilled person to reproduce the subject-matter of claims 17 to 26 in the absence of any information with respect to suitable cell lines.

3.3.1.4 According to **D19**, the authors then used a commercially available cell line of human colon carcinoma. Using oligonucleotides based on the information of the N-terminal sequence, 4 clones were detected in a λ-gt11 library from human colon carcinoma, but none of them contained a translation initiation start or stop codon. The sequence information of one of these clones, designated C2, allowed the construction of a second probe. However, rescreening of the

previously used human colon carcinoma cDNA library using a sequence derived from C2 was unsuccessful (**D19**, page 3270, left column):

> "Rescreening the colon cDNA library using another probe corresponding to a sequence found in C2 (see materials and methods) yielded several other recombinant phages containing inserts that overlap with the C2 insert. However, <u>none of them provided further sequence information on the cDNA in the 5' or the 3' directions.</u>
>
> *(emphasis added)*

The failure to detect the full length gene in the human colon carcinoma library thus necessitated screening of even further libraries. We refer to the "Thesis for the Degree of Doctor of Philosophy" by Oliver Kemper **(DA37)** cited as an expert's opinion, which was submitted to the Scientific Council of the Weizmann Institute of Science, Rehovot, Israel in November, 1994. On page 29 of this Thesis, there is the supplemental information that using oligonucleotide C2, a placenta library was screened:

> "In order to obtain more complete cDNA clones, we screened placenta library with the labeled 970 bp cDNA insert as a probe. Four positive phages were purified, two of which consisted of 2 Eco RI fragments each. The two shorter fragments (800 and 700 bp) seemed to be unrelated to the C2 clone, since they did not hybridize to the same band in Northern analysis as did the original 970 bp C2 probe. The two other phages, termed P3 and P4, contained inserts of 2 kb (kilo base pairs) each. P4 was partially sequenced. At the 3' end, we could identify a poly A sequence. In the 5' end, the clone showed a stretch of about 100 nucleotides identical to the sequence found at the 3' end of C2, after which the homology to the C2 sequence stopped and an apparently unrelated sequence followed. The 3' part of this clone contained repetitive sequences. Comparison of the restriction map and the localization of repetitive elements in the P4 clone and the p55 genomic subclone G1-hindIII led us to the assumption that the P4 clone was derived from the p55 gene, but unspliced and moreover, transcribed in the opposite direction (see Fig. 1)."

Thus, screening of this library was also unsuccessful. The proprietor finally was able to isolate the full length gene in a λ-ZAP library from mRNA of CEM lymphocytes, obtained from Clontech (**D19**, p.3270, left column). However, this λ-ZAP library from CEM lymphocytes **was not available at the European filing date**. We enclose copies of relevant pages of the Clontech catalogue 1988 **(DA38)** to demonstrate, that the library allowing to isolate the full length gene is

not offered at that time. We further enclose copies of the 1989/1990 edition **(DA39)**, including a reference to the CEM lymphocyte cell line used by the authors of **D19**. According to information obtained from Clontech the library was first listed in **DA39**, which was initially released in July/August, 1989. First release to third party was a few months earlier, i.e., in April, 1989.

From the above it follows that even the proprietor would not have been able to indicate a suitable cDNA library at the time of filing the opposed patent. In view of the failure of the competitive teams and in view of the failures admitted in **D19**, it must be concluded that the selection of a suitable library is most critical. Certainly, selection of the appropriate library was an undue burden at the European filing date. If any, then the above examples make clear that there was absolutely <u>no certainty</u> in reproducing (or, more exactly: producing) the claimed invention. Therefore, the claimed recombinant embodiments are clearly not sufficiently disclosed.

3.3.2 <u>Availability of specific antibodies (Section 5.3.2.1 of Proprietor's letter)</u>

The Proprietor alleges that the patent would enable the preparation of specific antibodies either by using the "substantially purified TNF inhibitor protein" or by using synthetic peptides. This is not correct.

3.3.2.1 As is admitted by the Proprietor and obtainable from Figure 6, the protein obtained according to the proposed patent is heavily contaminated with other proteins, thus rendering the preparation of specific antibodies almost impossible.

In this context, it should be noted that none of the several publications authored by the inventors and disclosing antibodies (<u>all post published</u>) relies on a preparation obtained according to the opposed patent for preparing antibodies. Rather, for preparing antibodies <u>affinity purified</u> TNF inhibitors are applied. It is referred to **D20**, page 1532, disclosing under the heading "Purification of the TNF-binding proteins" a process comprising an affinity chromatography step (ligand: rhuTNF-α) and reversed phase HPLC. This disclosure supports our argument that antibodies having a specificity sufficient for screening libraries

could not have been prepared with the admittedly impure preparation provided by the patent.

Moreover, the patent does not (and could not) offer affinity purification as an alternative option to purification. Reference is made to EP412486 (**DA40**) cited as an expert's opinion. **DA40** has an earliest priority date of August 6, 1989 and it states the following:

> "The protein TBP-I was first described in our European patent application EP 308,378 published on March 22, 1989, in which was disclosed a process for its purification to homogeneity from human urine by chromatography on CM-Sepharose followed by high performance liquid chromatography (HPLC) on Mono Q and Mono S columns and reversed phase HPLC. The homogenous TBP-I thus obtained had an apparent molecular weight of about 27,000 in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) under both reducing and nonreducing conditions. Homogeneity of the purified protein was confirmed by microsequencing analysis which revealed a single N-terminal sequence: Asp-Ser-Val-Cys-Pro. TBP-I was shown to protect cells from TNF toxicity at concentrations of a few nanograms per ml and to interfere with the binding of both TNF-α and TNF-β to cells, when applied simultaneously with these cytokines. <u>Further examination</u> of the mechanism by which TBP-I functions <u>revealed that TBP-I</u> does not interact with the target cell, but rather <u>blocks the function of TNF by binding TNF specifically</u>, thus competing for TNF with the TNF receptor.
>
> Consequently to this finding, we attempted an alternative approach for the purification of TBP-I, whereby urinary proteins or fractions thereof were applied on a column of immobilized TNF and, after removal of unbound proteins, the proteins which bound to the column were eluted, in bioactive form, by a decrease of the pH."
>
> *(emphasis added)*

The opposed patent refers to the protein as TNF-inhibitor, not TNF-binding protein. From a publication by one of the inventors we learn the following:

> "The ability of the purified TNF inhibitor to bind to several cytokines was examined using radiolabeled preparations of the purified protein in a solid phase assay... IL-1 or IFN-γ did not compete for the binding of the protein which protected against TNF cytotoxicity bound to the cytokine itself. Therefore the protein was termed TNF binding protein".
> (**DA48** on page 41)

From these quotations it becomes clear that at the priority date or European filing date even the proprietor lacked any basis for providing the purification

procedure which proved to be essential for further progress, i.e., affinity purification.

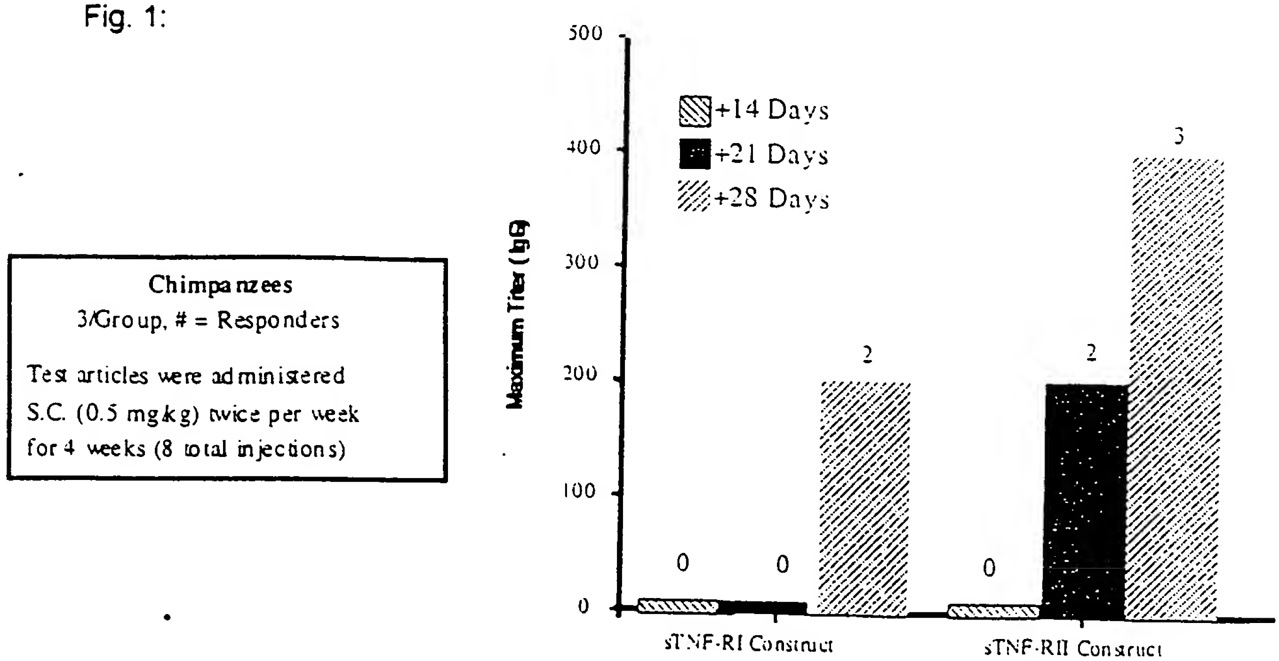
The requirement to know the binding capability of TNF-bp I is also supported by the proprietor's statement in his letter dated August 22, 1996, arguing against **D16** as an enabling reference since

> "Seckinger (**D16**) did not even disclose that the factor can bind TNF (such information would be required to developing an affinity chromatography purification step)."

3.3.2.2 There are a number of proteins being at least as immunogenic as TNF-bp I which, given the purification scheme of the proprietor, would co-purify; see Section 3.2.3 above. Considering the immunogenicity of these proteins a person skilled in the art would expect a laboratory mammal preferentially to develop antibodies against TNF-bp II rather than against TNF-bp I. In an experiment conducted by the opponent, it could be shown that in chimpanzees TNF-bp II is much more immunogenic than TNF-bp I, as will be detailed below:

Similar constructs of sTNF-R I and sTNF-R II were injected into chimpanzees. As demonstrated in the following diagram (Fig. 1), the maximum titer (IgG) in chimpanzees is markedly higher upon injection of TNF-R II construct than upon injection of the TNF-R I construct.

Fig. 1:



Thus, in view of the contaminants admitted to be present in the "substantially purified" preparation, antibodies are likely to be elicited against contaminants at least to the extent that they create a lot of false positive results in immuno screenings.

Considering further that the patent provides the sequence of the N-terminal sequence only, each of these clones would have to be sequenced in order to confirm the presence of the amino terminal sequence. Given the fact that TNF-bp II is likely to be one of several contaminants, this approach is clearly beyond the border of a reasonable amount of trials and errors (T 226/85).

3.3.2.3 The proprietor repeatedly attempts to argue in favour of the purity of the protein and alleges that the purity would have allowed to determine the whole amino acid sequence. On page 5 of the proprietor's letter dated August 22, 1996, it is alleged:

> The TNF-inhibitory protein provided by the present invention is pure enough to determine the whole amino acid sequence (which is the pre-requisite for the development of a system for recombinant production of the protein). However, as anyone skilled in the art knows the sequence of the N-terminal portion of the protein is determined easiest and first, which is also the case here. The rest of the sequence was also determined in the same way, but after the filing date of the EP application.

3.3.2.3.1 Determining the "rest of the sequence" in "the same way", i.e., by sequential analysis of the complete protein, is certainly not possible. Even nowadays routine analysis of purified proteins usually does not yield more than 30 to 40 amino acids along the primary sequence.

In fact, in **DA26** (Olsson et al., Eur. J. Haematol. 42: 270-275, 1989), the authors report purifying TNF-bp I 1,000.000 fold, and yet only report obtaining the first 20 amino acids.

3.3.2.3.2 The post published literature introduced by the proprietor demonstrates clearly that even by August 4, 1989, which is the date of submission of **D20**, sequences exceeding the N-terminus apparently were not available to the proprietor. It was only by April 26, 1990, when the proprietor was able to

provide sequences, but again not by step to step sequencing. Rather, the proprietor produced tryptic digests of affinity purified material, which finally permitted to obtain additional sequence information.

When it was so easy to determine the amino acid sequence, which, as correctly stated, is a pre-requisite for the development of a system for recombinant production of protein, why did it take so long? And why should the European Patent Office grant a patent upon something which admittedly was not available (that means not sufficiently disclosed), at the European filing date?

3.3.2.4 Assuming for the sake of the Proprietor's argument that eliciting a specific antibody towards TNF-bp I would have been possible (which is denied), there is still no certainty that the antibody would be specific for TNF-bp I. As is now an established fact, TNF-bp I is the TNF binding domain of a larger molecule called TNF-receptor I. TNF-receptor is one member of the type 1-transmembrane TNF-NGF receptor superfamily **(DA41)** (Gruss & Dower, Blood 85: 3378-3404, 1995; page 3378 submitted herewith). This family is now acknowledged to include at least ten proteins characterized by multiple cysteine-rich domains in the extracellular region. As shown in **DA41**, the average homology in the cysteine-rich extracellular region between the human family members is in the range of 25 % to 30 %.

In view of the conserved extracellular region, a search for extracellular human receptor sequences exhibiting sequence homology with TNF-bp I was done. The search was designed so as to detect sequences from the extracellular part of human receptors containing at least five contiguous residues homogenous to those of sTNF-R I. Various studies indicate that the size of an epitope that combines with an antibody is approximately equivalent to 5-7 amino acids [Benjamini et al., in Immunology: a short course, 3rd Edition 1996, Wiley-Liss, Inc., New York (**DA42**)]. In addition, the homologous human receptor sequences that are within the TNF-NGF family were further analyzed in an attempt to predict if the homologous regions to TNF-bp I would be accessible to cross-reacting antibodies when those extracellular regions would be in a native folded state. The data of this search and its results are submitted as **Annex 4**.

Summarizing the results set out in **Annex 4**, TNF-bp I shares exposed residues with CD40, CD27, TNRC, CD30, TNF-R II, NGF-R and OX40. Thus, there are at least seven further proteins which are likely to cross-react with any polyclonal serum raised by immunizing mammals with mature purified TNF-bp I. Given the fact that e.g. TNF-bp I and TNF-bp II are co-expressed, given further the fact that TNF-bp II appears to be more immunogenic than TNF-bp I (see section 3.3.2.2) and that similar results cannot be excluded for any of the other members of the TNF-NGF receptor family, it would amount to an enormous amount of work to identify clones expressing TNF-bp I or cells carrying TNF receptor molecules.

Thus, even if the proprietor would be successful in producing specific antibodies using the preparations disclosed in the opposed patent (which is denied), it is <u>not at all certain</u> that these antibodies would be suitable to identify TNF-bp I in other cells or to identify recombinant clones expressing the protein.

3.3.2.5 Finally, with respect to the possibility of using synthetic peptides, this is also a very uncertain approach for the following reasons.

3.3.2.5.1 Short oligo peptides do not necessarily fold the same way they would fold when attached to their native protein. In the present case, it is even highly unlikely that the N-terminus would fold as in the native protein: In TNF-bp I, Cys^4 forms an S-S bridge with Cys^{19}. In a synthetic peptide based on the disclosure of the opposed patent, there is no Cys^{19}.

In the absence of this disulfide bridge constraint the secondary and/or tertiary structure of the synthetic peptide is very likely to be random and not necessarily similar to the native structure of TNF-bp I.

3.3.2.5.2 It is known that short peptides do not necessarily possess a defined tertiary structure. A conformational study was done to determine if peptide #2 (described in Section 3.3.2.5.3, below) would be expected to maintain a stable conformation. The study utilized molecular dynamics simulation techniques to probe the conformational variability of peptide #2. Molecular dynamics simulation of peptide folding is an accepted methodology [Sung and Xiong-

Wu, Proteins 25:202-214, (1996) cited as an expert's opinion (**DA43**)]. In an effort to give the peptide its best chance of adopting a stable conformation, the simulated peptide was initially placed in a conformation that has been reported for sTNF-R I [Naismith et al., Structure 4(11):1251-1262, (1996), cited as an expert's opinion (**DA44**)]. During the course of the computational simulation the simulated peptide unfolded and predominantly adopted a random, somewhat extended conformational state. There was much fluctuation and movement of the peptide conformation during the simulation. Thus, it would be expected that the synthetic peptide would not produce a very specific antibodiy response.

3.3.2.5.3. In order to test whether or not the synthetic peptide approach may be feasible, two peptides derived from the N-terminal part of the protein were synthesized, i.e.,

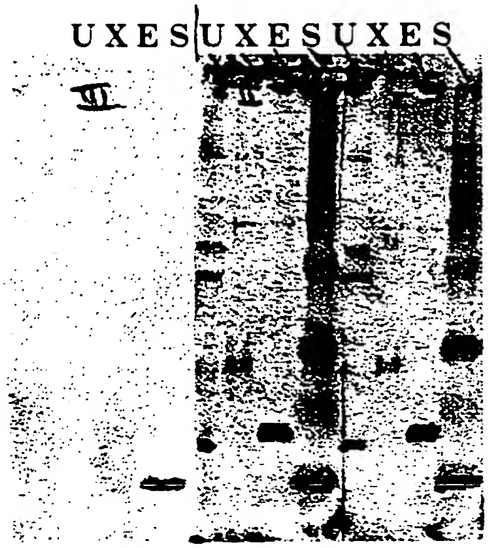
#1:CPQGKYIHPQ
#2:DSVCPQGKYIHPQN.

After the simulation experiment, these peptides were used to immunise rabbits, and the specificity of the thus obtained polyclonal antibodies was examined. All experiments were carried out using the techniques and detection systems which were available in 1988 [with the exception of using *E. coli* derived sTNF-R I (prepared substantially in accordance with the teachings of EP422339 (**DA45**)) in the competition experiment outlined below].

When incubating the polyclonal antibodies with samples of human urine, the antibodies elicited by both the above given synthetic peptides reacted predominantly with five proteins in the molecular weight range of 15 to 90 kD; in addition, a large number of proteins were weakly stained. A copy of the Western blot is provided in Fig. 2 below (the original will be available at oral proceedings).

Fig. 2:

Western blot using rabbit anti-N-terminal peptide antibodies.



Triplicate of lanes were prepared. One set (indicated as I in the figure) was for a normal western blot using rabbit anti-N-terminal peptide antibody. Another set (II) was for a western blot in the presence of a competitor and the other set (III) was for a western blot control with only second antibody (anti-rabbit IgG peroxidase). Each set contained pre-stained molecular weight standard (2 μg), 30 ng of *E. coli* derived sTNF-R I and 10 μg of concentrated human urine. 14% SDS-PAGE system was used. Lane S is MW standards, lane E is *E. coli* derived sTNF-R I and lane U is human urine.

Thus, polyclonal antibody #1 is highly non-specific.

Antibodies to peptide #1, were more reactive to healthy human proteins than antibodies to peptide #2, and were chosen for a further experiment. In this experiment, specificity of the antibody was studied using a competition assay. A 5,000 fold dilution of antibodies to peptide #1 were incubated with a Western blot of urine in the presence of excess competitor, i.e., *E. coli* derived sTNF-R I **(DA45)**. In the case of a specific antibody, one would expect the band pattern of

the Western blot to be different upon incubation in the presence or absence of competitor. However, as is shown in Fig. 2 above, the Western blot obtained in the presence of a 10,000 fold excess of competitor looks very much the same as the one obtained in the absence of competitor.

3.3.2.5.4 In addition to this non-specificity, rendering it unfeasible to apply polyclonal antibodies elicited by synthetic peptides to identify a suitable cell source, this approach was also technically unaffordable at the relevant date. The use of a more or less specific antibody requires the confirmation of the results by competition experiments. In the present case, more than a 10,000 fold excess of competitor would be required; this means that in order to detect and identify 30 ng of specific TNF-bp I, which is the average amount of a protein band in an SDS-PA gel, it will require 300 μg or more of TNF-bp I competitor. To purify 300 μg of TNF-bp I from human urine in compliance with the patentee's process, more than 12000 liters of human urine would be required (according to the patent, 5 μg or less was purified from 200 liters).

It should be noted that a synthetic peptide could be used as competitor. However, since the anti-peptide antibody interacts with many proteins, purified TNF-bp I is the appropriate competitor. Thus, 300 μg of TNF-bp I would be required.

3.3.2.6 The applicability of a polyclonal or monoclonal antibody against synthetic peptides for the identification of a cell source for TNF-bp I by screening cells or clones expressing p55 TNF-receptor type I is furthermore questionable in view of the glycosylation site at the N-terminus. One of the TNF-bp I glycosylation sites is an Asn^{14}.

Generally, the termini of proteins belong to the more immunogenic portions of the molecule. However, the antigenicity may be reduced or at least influenced by glycosylation sites. Thus, sugar moieties may mask an epitope on the mature human protein which is recognised by antibodies to *E. coli* derived material. Moreover, it is well known in the art that the glycosylation patterns differ not only from one human individual to another human individual, but that glycosylation in different eukaryotic cell systems leads to distinguishable glycosylation patterns.

Thus, a protein (or peptide) produced in COS cells is different from a protein (or peptide) produced in CHO cells or produced in yeast cells, and each is different from a protein produced in human cells. Consequently, the N-terminus of the native human protein may have a conformation different from the peptide or the peptide's epitope may be masked by the sugar moiety.

3.3.2.7 Furthermore, even if antibodies should be obtained, their use for determining suitable cell lines is highly questionable. This is demonstrated e.g., by **D20**. As discussed above, **D20** applied affinity purified material to produce antisera. An anti-TNF-bp I antiserum was used to study its inhibitory effect on TNF binding to different cell lines. The experiments were assumed to "reflect the interaction of the antibodies with the receptors resulting in interference with the binding of TNF to them" (page 1534, right column, first paragraph).

Curiously enough, the authors could not show any inhibitory effect of anti-TNF-bp I antibodies when same were added to U937 cells, thus allowing the conclusion that U937 cells do not contain an mRNA encoding membrane bound TNF-receptor. This result is clearly wrong as can be obtained from e.g. **DA46** (Schall et al., Cell 61: 361-371, 1990), cited as an expert's opinion . According to **DA46:**

> Uninduced U937 cells were found to have relatively high levels of TNF-R mRNA, (*Page 364, left column)*

Thus, the approach suggested by the patentee is not only highly unreliable in view of patentee's own results published in **D20**, it even may be misleading.

3.3.2.8 As a side note it shall be added that the cell line which proved to be suitable for isolating the TNF-bp I coding DNA, i.e., CEM lymphocytes, would not have been selected on the basis of any antibody screening. It is referred to the Kemper Thesis **(DA37**, page 33/34) depicting on page 34 in Table 1 the influence of cytokines on ^{125}I-TNF binding. The left column of the treatment section (headline: control) provides information on the relative amount of TNF-bp receptors in uninduced cells. As may be obtained from said column, CEM, having a value of 440+/- 12, displays the second smallest amount of bound TNF

from all cell lines investigated. This is certainly not encouraging for using this very cell line for establishing a cDNA library.

3.3.2.9 Furthermore, as already disclosed above, TNF-bp I is a member of a receptor family having partially conserved domains. This family is now acknowledged to include at least ten different receptors. **D19** demonstrates nicely that in particular amino acids 20 to 35 of TNF-bp I (see Figure 2 of **D19**), corresponding to amino acids 1 to 16 of the patent, i.e. the N-terminus of the mature protein, share considerable homology with TNF-bp II, NGF-R and CD40. Therefore, even if the short peptides would act as immunogens as they are supposed to do by the Proprietor, the antibodies elicited by these oligopeptides are likely to be unable to discriminate between related proteins belonging to the same receptor family. Consequently, immunizing with short oligopeptides is also not an appropriate approach.

3.3.3. <u>Use of oligonucleotide probes (Section 5.3.2.2 of Proprietor's letter)</u>

According to section 5.3.2.2 of the Proprietor's letter, the patent is enabling for the preparation of a cDNA library and for screening of said library in order to obtain the gene encoding TNF inhibitory protein. However, the Proprietor's arguments are not based on proper facts.

3.3.3.1 As outlined above, there was no known source for preparing a cDNA library (see section 3.3.1).

3.3.3.2 The disclosure is not sufficient for selecting a genomic clone for a variety of reasons.

Firstly, it is well known that libraries may have holes. This applies in particular to genomic libraries, wherein a certain gene may span several tenths of kilobases due to the presence of introns. In this context, it is referred to **T412/93** (Erythropoietin/KIRIN-AMGEN), wherein an Appeal Board explicitly acknowledged that genomic libraries are not necessarily complete (see point 142(ii) of that decision). Thus, the likelihood that at

least part of a gene is not contained in a particular library, is extremely high.

Secondly, most genomic copies of genes possess introns, the boundaries of which need to be determined. Although there are certain known splice donor and splice acceptor sites, the location of splice cites can not be determined by sequencing alone since (a) sequencing even nowadays often is a cumbersome task, which certainly at the European filing date would have required an undue burden, and since (b) the interpretation of the sequences is not unequivocal. Therefore, the boundaries between exons and introns need to be determined or at least confirmed by a corresponding cDNA sequence. Since this was not enabled, the genomic DNA also was not.

Thirdly, in the absence of any information relating to introns or exons it is not even possible to determine the C-terminus, which was also not disclosed in the opposed patent. Besides the proprietor only disclosed a glycosylated protein of 26-28 kD,whereas the genomic clone would encode the full length receptor. Consequently, there is absolutely no room for assuming that a genomic DNA would be sufficiently disclosed.

3.3.3.3 Finally, the amino acid sequence disclosed in the patent is not sufficient for preparing oligonucleotides successful in detecting desired cDNA or genomic clones. This view is supported rather than contradicted by **D19**, attempting in 1990(!), i.e. two years later than the European filing date, to clone the TNF-bp I gene.

As will be immediately apparent from reviewing **D19**, providing a cDNA sequence comprising the complete TNF-bp I coding region was a very complex process scattered with uncertainties.

The uncertainties contributed by the selection of a suitable cDNA library have already been discussed above. But also the design of a probe enabling the isolation of the full length clone is not as simple as the proprietor intends to make believe.

3.3.3.3.1 Allegedly, the post-published reference **D19** relates to the successful cloning of TNF-bp I following "exactly the approach" disclosed in the patent (proprietor's letter of August 22, 1996, page 17, second paragraph).

It was already discussed above, that this statement is simply not true, since the success of the approach published in **D19** depends on the availability of a CEM cDNA library as well as on the availability of probe C2. Further, it is noted that **D19** reduces the degeneracy of the probes applied for hybridization by including inosine (**D19**, page 3277, Section "Isolation of cDNA clones"). The patent, however, neither explicity nor implicitly suggests use of inosine. The teaching given in the patent concerning probe design is limited to the following (page 10, lines 46 to 49):

> "This oligonucleotide containing such a complementary sequence may then be synthesized and employed as a probe to identify and isolate the gene of the TNF-inhibitory protein of the invention (Maniatis T. et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982)."

We have checked the Maniatis 1982 issue and submit that the choice of inosine is not discussed in this book. The same applies to any of the further references cited in the cloning context in the patent, i.e., Lathe et al., Hames et al., Pennica et al..

Thus, the patent does neither explicitly nor implicitly teach the use of inosine.

However, in view of the highly degenerate N-terminus, it was necessary to incorporate inosine at the positions where all 4 nucleotides were possible in order to keep the degeneracy at an appropriate level. With inosine the probe of D19 was 48X degenerate, without it would have been 3072X degenrate. 3072X decreases the concentration of the correct probe in the pool so far that the probe would not be useful.

It is also apparent in the discussion of the placental library screening in **DA25** that the 3 probes non-specifically hybridized to clones that did not encode

sTNF-RI. This further compounded the difficulties in identifying a clone encoding sTNF-RI.

The use of inosine is just a further deviation from "exactly the approach" taught in the patent.

3.3.3.3.2 Nophar et al (**D19**) in fact initially used oligonucleotides based on the N-terminal sequence disclosed in the patent. According to **D19**, a commercially available human colon carcinoma cDNA library was screened with a set of oligonucleotides based on N-terminal sequence information and yielded 4 recombinant clones having inserts similar in size and found to overlap by restriction mapping and sequence analysis. As mentioned above none of these clones contained a translation start or stop codon. The thus obtained sequence information permitted to construct another probe, designated C2. It was the combination of this probe C2 (which is not based on the N-terminal amino acid sequence), and a library available only subsequent to the European filing date which enabled the proprietor to provide the full length gene.

3.3.4 Lack of sufficient disclosure of claims 18 and 23 and claims depending thereon

With respect to expression in prokaryotic hosts, the patentee alleges that a person skilled in the art, having obtained the cDNA, could easily modify the cDNA in such a way that its expression leads to the production of the soluble protein, for example by deleting by site directed mutagenesis the region of the cDNA which encodes a transmembrane region. According to the patentee's submission, once having determined the C-terminus of the protein, the transmembrane region can be determined by hydropathy index computation according to **D22** or **D23**.

It is submitted that these statements lack a logical basis and ignore the pecularity of TNF-bp I.

3.3.4.1 Firstly, as already discussed above, the patent does not enable the cDNA. Thus, in the absence of a cDNA a cDNA can not be modified.

3.3.4.2 Secondly, the patent also does not disclose the C-terminus of the protein. It neither provides the C-terminus of the truncated protein actually disclosed nor of the full length TNF-bp I nor of the complete TNF-receptor.

3.3.4.3 The proprietor is mistaken if he believes that hydropathy index computation would provide the correct results. The TNF-receptor coding DNA exhibits several pecularities, which are not disclosed in the patent and also can not be interpreted to be disclosed. For example, the first 29 amino acids of the coding region form a signal sequence. The amino acid of the mature TNF-receptor thus starts with amino acid 30 of the coding region, i.e., Leu^{+9} according to Fig. 1D of **D19**. The N-terminus of TNF-bp I, however, is located 11 amino acids further downstream, i.e., Asp^{20} according to **D19**. While the patentee might have been capable of determining the correct N-terminus, the situation is quite different with respect to the C-terminus. According to the numbering system of **D19**, the complete TNF-receptor consists of 434 amino acids. The transmembrane region starts with Val^{191} (see the round ended box in Fig. 1D of **D19**). This is the boundary a hydropathy computation index would have provided. However, as is also obtainable from Fig. 1D of **D19**, this is not the actual C-terminus of TNF-bp nor is it the C-terminus of the truncated form provided by the patentee. TNF-bp extends from Asp^{20} to Asn^{180} rather than to Thr^{190}. This information is only available upon having determined the C-terminal amino acid sequence (see **D19**, page 3276, second paragraph of "Materials and methods"). This, however, requires provision of full length TNF-bp I, which was not available according to the process disclosed in the opposed patent. Therefore, determining the correct C-terminus is not possible on the basis of the patent.

3.3.4.4 In addition, it is submitted, that the opponent's attempts to express full length TNF-bp I in *E. coli* have shown that the protein is expressed in inactive form. In order to obtain active TNF-bp I, the protein must be isolated, reduced and allowed to refold in an appropriate manner. Since the requirements for optimal unfolding and refolding heavily depend on the nature of the protein in question, this is another difficulty which would place an undue burden on a skilled person attempting to reproduce recombinant expression in prokaryotic host.

Summarising the above, expression of TNF-bp I in prokaryotic cells is not enabled for even more reasons than expression in eukaryotic hosts.

3.3.5 Relevant Case Law

As a concluding consideration, the sufficiency of disclosure of the DNA related embodiments shall be discussed with a view to case law issued by the Technical Boards of Appeal, in particular by Technical Boards of Appeal responsible for biotechnological inventions.

3.3.5.1 Generally, a disclosure must be reproducible without undue burden. This principle has been established, e.g., by T226/85 (OJ 1988, 336) where the Board considered that even though a reasonable amount of trial and error is permissible, e.g. in an unexplored field or where there are many technical difficulties, a skilled person had to have at his disposal, either in the specification or on the basis of common general knowledge, adequate information leading necessarily and directly towards to success through the evaluation of initial failures. These principles have been confirmed by former TBA 3.3.2 and by TBA 3.3.4 in a number of decisions, two of which will be mentioned below.

3.3.5.2 In the patent underlying decision **T418/89** (Monoclonal Antibodies/ORTHO) the following claim had issued:

> "Mouse monoclonal antibody which (i) reacts with essentially all normal human peripheral T cells, but (ii) does not react with any of the normal human peripheral cells in the group comprising B cells, null cells and macrophages."

According to the specification, the hybridomas producing a claimed monoclonal antibody could be produced by using E-rosette positive purified normal peripheral T-cells as the antibody stimulating antigen. When considering sufficiency of disclosure, the Board held:

> "However, this fact alone is not sufficient to make the process reproducible as to monoclonal antibodies having the characteristics of claim 1. To select a hybridoma of the desired kind in any case means a huge amount of effort and, above all, *it is not certain* that this hybridoma can be selected at all. (Point 3.4 (emphasis added))"

It should be noted that <u>certainty</u> for obtaining the desired result (which in T418/89 is the hybridoma and which in the present case is a cDNA) is required. The TBA went on and held:

> "The Board considers that in the circumstances of the present case, where the written description of how to produce a hybridoma is basically a known cumbersome and random general process and a specific technical teaching is provided only by identifying the type of the antigen, being E-rosette positive purified normal human periphery T-cells, the requirements of Article 83 EPC are not met. (Point 3.7)"

Applying this statement to the present case means that since only basic processes for producing a DNA and a partial amino acid sequence are provided, this cannot be considered sufficient to acknowledge sufficiency of disclosure for a DNA molecule coding for the complete amino acid sequence.

3.3.5.3 Even more relevant may be **T412/93** (Erythropoietin/KIRIN-AMGEN). In that case sufficiency of disclosure of a cDNA was an issue. Table VI of the patent in dispute provided a DNA sequence; however, said table gave no information as to where the cDNAs are supposed to start or to stop. Consequently, the Board held that:

> There is thus no unambiguous identification of any sequence in the patent as human cDNA. (Point 21)
>
> To identify a partial sequence on Table VI as being the cDNA would be mere guess work. Neither is there an unambigous information of the start nor an indication of where the end should be. (Point 28).

Therefore, although in that case a DNA sequence was provided, the Board did not acknowledge sufficiency of disclosure for a claim relating to a cDNA depicted in that application, because neither translations start nor stop codons were indicated.

Compared to that case, the present one suffers not only from the fact that the proprietor did not indicate a start or stop codon - it suffers already from lack of a cDNA related sequence. Looking closer on the case it becomes apparent that

there is not even a protein sequence corresponding to the claimed cDNA sequence!

Therefore, applying the principles of T412/93 as expressed in points 21 and 28, there can be no doubt as to the fact that there is no sufficient disclosure with respect to a cDNA sequence.

The Board then investigated whether as a means for preparing cDNA, a reliable method for obtaining mRNA was disclosed. Since no source which expressed erythropoietin was available, sufficiency of disclosure for a cDNA was not acknowledged. This part of decision T412/93 can be directly applied to the cDNA claimed in present claim 19 et seq., because also in the present case, there is no indication whatsoever which cell line should be used in order to isolate an mRNA.

Furthermore, in T412/93 the sufficiency of disclosure of a genomic DNA was further discussed, in particular the necessity of a deposit. The Board held

> "This need for a deposit cannot be introduced by reference to the concept of undue burden. This concept relates more to cases where the route that the reader is to follow is so poorly marked that <u>success is not certain</u>. (Point 76)*(emphasis added)*

Consequently, if there is a clear teaching how to obtain the desired result even in the absence of a deposit, a deposit is not required. However, a deposit is undoubtedly required, where <u>success</u> of obtaining a particular DNA <u>is not certain</u>. Since this is the case with respect to the opposed patent, and since the Patentee neither deposited a clone corresponding to the claimed DNA nor provided any information with respect to start or stop of the claimed sequence, the DNA sequence can not be deemed to be sufficiently disclosed.

3.4 Lack of sufficient disclosure of claims 27 to 30

Claims 27 to 29 relate to pharmaceutical compositions containing the protein according to any of claims 1 to 10, 16 to 18 or 26 and claim 30 relates to the use of these proteins for preparing a pharmaceutical composition. Since these

claims are directly or indirectly dependent on claims 1 to 3, 16 or 18 to 26 they are insufficiently disclosed for the reasons discussed above in Sections 3.1, 3.2, 3.3 and 3.4.

3.5 Lack of sufficient disclosure of claims 31 to 33

Claims 31 to 33 relate to antibodies. Due to the insufficiency of disclosure for claims 1 to 3, 16 and 18 to 26 they are also insufficiently disclosed. In addition, the antibodies are not enabled for the reasons given above in Section 3.3.2 (3.3.2.1 to 3.3.2.5).

4. <u>Lack of Novelty (Art. 54 EPC)</u>

4.1 Lack of novelty of claims 1 and 2 over D7

When discussing **D7**, the propdetor alleges that

(i) **D7** does not disclose the isolation and purification of a receptor in an enabling manner

(ii) **D7** does not disclose that the allegedly non-enabled substances have a biological activity as recited in the patent

(iii) The TNF receptor disclosed by **D7** is assembled to TNF and therefore can not have the biological activities recited in claim 1

(iv) It stands to reason that subsequent to dissociation yielding free labelled TNF-α other components remained, e.g., cells with the cell bound TNF receptors, and not an isolated TNF receptor.

However, the proprietor's arguments are not convincing.

4.1.1 <u>The purification process</u>

D7 discloses the purification of TNF receptor by the following steps:

(a) Incubation of cells for 2 hours at 4°C with ^{125}I-TNF-α in PBS-FCS
(b) Washing of cells
(c) Resuspension in PBS containing DSP
(d) Incubation for 20 minutes at 4°C
(e) Washing of cells in Tris-buffered saline
(f) Suspension in buffer containing non-ionic detergent
(g) Incubation at 4°C for 1 hour
(h) Centrifugation for 5 minutes at 500 x g
(i) Centrifugation for 1 hour at 100,000 x g
(j) Precipitation of protein from supernatant by addition of cold acetone
(k) Dissolving precipitate in buffer containing SDS
(l) Separation of proteins by SDS electrophoresis in a 7.5% PA-gel in the absence or presence of mercapto ethanol.

The purification process as described above is different from that disclosed in the opposed patent, but it is not necessarily less effective. It should be recalled that the protein according to granted claim 1 is not required to have any specific purity. Furthermore, the protein of claim 2 is obtained by lectin affinity chromatography and gel filtration <u>or</u> ion exchange chromatography. This process apparently does not yield single bands but rather a relatively crude preparation as evident by Fig. 6, lines B, C or D of the opposed patent. **D7** discloses a TNF-inhibitor having at least the same degree of purity and having the ability to bind TNF.

4.1.2 **D7** discloses without question that TNF receptor binds TNF. This is mentioned, although it is considered not to be necessary to demonstrate any biological effect at all. Attention is drawn to the case law of the Appeal Boards of the European Patent Office, according to which it was repeatedly stated that the characterisation of a certain compound by further parameters, e.g., further activities, does not render an already known compound novel. In the present case, if a tumor necrosis factor inhibitory protein, i.e., the TNF receptor of **D7**, is already known, it is totally irrelevant whether or not the prior art disclosure was

able to indicate certain biological activities, which are inherent to the protein and thus are a feature of the protein whether explicitly mentioned or not.

4.1.3 **D7** has demonstrated that cross linking of TNF and TNF receptor using DSP is reversible and that reacting the complex comprising TNF receptor and TNF with β-mercapto ethanol liberates TNF-α. As will be apparent to any person skilled in the art, it then also liberates free TNF receptor. If the Opposition Division should have any doubt as to this primitive chemistry, the opponent will be pleased to provide a respective expert's opinion. However, since it is a very trivial fact that the reversability of cross linking events using DSP sets free the compounds previously subjected to cross linking, this is not deemed to be necessary.
In contrast to the patentee's allegation, the material obtained after reversion of cross linking is still active. In Annex 3, an experiment relating to cross linking of TNF-bp I and a fluorescent marker is reported. The cross-linked TNF-bp I was still highly active.

Finally, it is submitted that the isolation of TNF-bp from an SDS-PAGE does not affect its activity. This is supported by **DA47** (Brockhaus et al., Proc. Natl. Acad. Sci. USA, 1987: 3127-3131, 1990), cited as an expert's opinion. As may be obtained from Fig. 5 and the corresponding description, TNF-bp separated by SDS-PAGE under non-reducing conditions is still capable of binding TNF-α. Thus, it still exhibits TNF-binding activity.

4.1.4 With respect to the allegation that after dissociation other components remained, i.e., cells with the cell bound TNF receptors, it is submitted, that subsequent to a 1 hour centrifugation at 100,000 x g there is certainly no cell remaining. Even if this should have been the case, it is submitted that the free TNF receptor is separated from other components in the gel during PA electrophoresis and thus it is pure at least to the extent the claimed protein is considered to be pure.

It is to be understood that to the question whether or not the disclosure of **D7** enables the provision of TNF receptor, the same criteria and standards should be applied as to the question whether or not the patentee was able to provide a protein rather than a protein composition.

4.2 Lack of novelty of claims 1 and 2 and dependent claims over D8 to D12

With respect to **D8** to **D12**, it is submitted, that each of these documents teaches the cross linking of TNF-α with TNF receptor and the separation of the membranes by SDS-polyacrylamide gelelectrophoresis. Therefore, each of these documents teaches to provide TNF receptor at least in a cross linked form purified to more or less the same degree as the proprietor teaches in the opposed patent. Therefore, we still consider each of documents **D8** to **D12** to destroy novelty of claim 1 and claim 2.

4.3 Lack of novelty of claim 3 and dependent claims over D16

The proprietor argues that

(i) **D16** merely enables for a heterologous mixture in contrast to the opposed patent, which provides a protein purified to such an extent that it can be sequenced as disclosed in Example 3.7 and

(ii) **D16** does not disclose that the factor can bind to TNF.

Both statements are at least misleading.

4.3.1 In the beginning, it shall be emphasised again that the protein disclosed by Seckinger et al., in **D16** is in fact the very same as that disclosed by the opposed patent. This is confirmed for example by **D21** cited by the proprietor, page 85, left column, first sentence of the last paragraph. The authors of **D21** cite 6 documents disclosing TNF-bpI and TNF-bpII, one of them being **D16**. This is further confirmed by **DA52** (Ythier et al., Cytokine 5: 459-462, 1993), citing on page 359 references 10 to 16, with reference 10 corresponding to **D16**. **DA52** further admits the identity of the claimed protein with the one disclosed in **DA53** (Peetre et. al., Eur. J. of Haematol. 41: 414-423, 1988). Thus, the proteins disclosed by Peetre, Seckinger and the Proprietor are the same.

4.3.2 Turning now to the proprietor's allegations, Seckinger admitted the presence of further bands in SDS-gels of the protein purified to a certain extent (as has the proprietor admitted the existence of higher molecular weight bands in its

material). Nevertheless, the protein has been purified, as will become obvious from the fact, that the inhibitory activity eluted from Sephracryl S200 gel filtration as a single peak. Comparing this language with that of the patent, it is noted that step 3.3 (cation exchange chromatography) and step 3.4 (anion exchange chromatography) conducted in compliance with the opposed patent result in elution profiies having a less unambiguous appearance, since in both cases it was only a major portion of the activity or the majority of the activity, which eluted in three to four fractions. It is only after HPLC that the opposed patent refers to sharply eluting activity. As we know by the submission of the proprietor, even this HPLC eluate does not contain pure TNF-bp I, which explains the fact that only 15 N-terminal amino acids could be sequenced. Therefore, from the mere comparison of the teaching of the patent with that of Seckinger it is not at all clear which of the obtained protein preparations is the purer one.

4.3.3 With respect to the allegation that **D16** did not reveal any parameter of the factor that could be used for further purification and accordingly there is no teaching for the person skilled in the art, it is submitted that **D16** of course provides an assay for the quantititation of TNF-α inhibitory activity. It is referred to page 1511 "Quantititation of TNF-α inhibitory activity". Figures 1 and 2 in addition demonstrate that this assay system was perfectly suitable for determining the active fractions.

In view of the fact that the patent lacks a definition of "substantially purified" (see our opposition brief, Section ...) which would be suitable to distinguish the claimed protein over the proteins of **D16**, the protein of **D16** anticipates the subject-matter of claim 3.

4.4 Lack of novelty of claims 4 to 10 and 16 over D16

Since, for the reasons provided above, **D16** in fact discloses a protein which is in substantially purified form, claims 4 to 10 and 16 are, in contrast to the proprietor's opinion, anticipated by **D16** for the reasons given in Section 4.3 above and in Section 6.2 and 6.3 of our opposition brief.

4.5 Lack of novelty of claims 31 to 33

The proprietor objects that it has not been shown for the antibodies disclosed in **D17** that they bind to the TNF binding region of the TNF receptor used for immunising animals. It is suggested that these antibodies bind to sequences upstream of the N-terminus or the linker between C-terminal end of TNF-bp I and transmembrane region.

In response to this suggestion, the following is submitted:

4.5.1 Usually upstream of the N-terminus there are no amino acid sequences.

4.5.2 The TNF-bp of the opposed patent does not have a specified C-terminus. Thus, it may also embrace the linker region and, consequently, antibodies might be directed to said linker region.

4.5.3 The proprietor apparently has overlooked the disclosure in **D17**, column 4, lines 30 to 36, and of Example 5 referred to in said paragraph. The quoted section from column 4 reads as follows:

> "If a monoclonal antibody of the present invention is bound to living cells carrying TNF-RI under physiological conditions the <u>cells lose their ability to bind TNF</u>. This will be better understood on the basis of the detailed description of the experimental conditions given in Example 5 hereinafter in connection with the accompanying drawings.
> *(emphasis added)*

From the fact that intact cells carrying TNF-receptor lose their ability to bind TNF-α it may safely be concluded that the antibodies according to **D17** bind to the TNF-binding domain. Since the TNF-binding domain is the same in TNF-receptor and TNF-inhibitory protein, the antibodies disclosed in **D17** clearly anticipate the antibodies claimed in the opposed patent.

It is not apparent, why the antibodies according to claims 31 to 33 should be regarded as selection invention.

5. Lack of an Inventive Step

5.1 Claims 1 to 10 and dependent claims

In item 7.1.3.1 of his letter the proprietor contends that the subject-matter of claim 1 is not obvious over Liao (**D3**) alone or in combination with Israel (**D7**) or Wallach (**D2**) for the following four reasons:

> Firstly, TNF-bp I and IL-1 inhibitor have different mechanisms of exerting activity. Therefore, a person skilled in the art allegedly would not expect to find TNF-inhibitory proteins in urine.
>
> Secondly, the purification methods are different.
>
> Thirdly, proteins can vary with respect to their structure, although having biological activities in common. Therefore, a person skilled in the art allegedly would not assume that the two proteins can be separated by the same fractionation techniques.
>
> Fourthly, allegedly none of the cited documents describes a suitable source for obtaining TNF-inhibitory proteins.

It is submitted that these arguments are without any merit for the following reasons:

5.1.1 Whether or not both the proteins in question, i.e., IL-1 inhibitor and TNF-bp I (TNF-inhibitor) exert their effects by the same type of mechanism is totally irrelevant for the question of inventive step for providing a TNF-inhibitory protein. In addition, these differences were not known at the priority date and thus certainly can not have prevented a person skilled in the art to transfer knowledge obtained with interleukin-1 inhibitor to TNF-inhibitor.

What counts in this situation are the following facts:

(i) There are two cytokines sharing several biological activities and physical features, i.e., IL-1 and TNF-α.

(ii) Both cytokines occur in urine.

(iii) The biological activity of both cytokines is subject to regulation by inhibitors.

(iv) The inhibitor for IL-1 is known to occur in urine.

This chain of facts necessarily leads to the idea that the inhibitor for TNF-α may also occur in urine! Evidence for this conclusion can be found in **D16, DA34** and in **DA48** ("Thesis for the Degree of Doctor of Philosophy" by Hartmut Engelmann, submitted with the Scientific Council of the Weizmann Institute of Science, 1992). In **D16**, it is stated (quotation from page 1511, second paragraph):

> "We previously isolated a specific IL-1 inhibitor from the urine of febrile patients (6-8). As TNF and IL-1 are both mediators of fever, we wondered whether such urine might also contain a TNF-α inhibitory (TNF-α INH) activity. Indeed, we found an inhibitory factor of 40 to 60 kD, therefore proving the existence, as in the case of IL-1, of at least one negative feed back regulator."

D16 is, subject to the decision concerning the relevant priority date for claims 1 and 2, likely to be post-published and thus cited as an expert's opinion with respect to claims 1 and 2. It nevertheless demonstrates that on the basis of facts, which were known before the priority date, conclusions can be and were drawn, which directly and without inventive effort lead to the subject-matter of claims 1 and 2.

DA34, authored by Wallach et al., discloses (quotation from page 360, second but last complete paragraph):

> "Trying to identify regulatory molecules which may contribute to the decrease in the responsiveness to TNF (7,8) we followed prior studies which indicated that the function of IL-1 can be suppressed by certain secreted inhibitors. Human urine has been shown to contain such inhibitors of IL-1 activity (15-17). We therefore examined whether the urine contains also components which can affect cell response to TNF. As

demonstrated in Fig. 1, concentrated preparations of the urinary proteins indeed suppressed effectively the cytocidal activity of TNF (18-19). A similar observation has been reported by others (20,21)."

DA48 discloses (quotation from page 30):

"It has been speculated for several years that human urine contains potent immunosuppressive factors. In particular the urine of pregnant women (147) and febrile patients (148, 149) has been examined extensively for its anti-inflammatory properties. This line of research led to the identification and purification of several low and high molecular weight components some of which antagonize in a very specific manner certain cytokines: An IL-1 inhibitor competing with IL-1 for its receptor binding site (150, 151) and uromodulin, a urine-derived glycoprotein, binding with high affinity to IL-1 and TNF (152, 153, 151) were the most prominent members of urine-derived immunosuppressive factors.

Following these findings urine samples of healthy donors and of patients suffering from different diseases were probed for the presence of TNF antagonizing factors."

(emphasis added)

DA34 and **DA48** are also post published documents. But again, **DA34** and **DA48** as well as **D16**, just verbalise a chain of thoughts created on the basis of facts known before the European filing date. Interestingly enough, references 7, 8, 15, 16, 17, 18, 20 and 21 of **DA34** are published in or before 1988.

5.1.2 With respect to the correspondence or non-correspondence of purification methods it is submitted that the methods clearly correspond as closely as methods established by different laboratories can correspond. The individual steps are essentially the same. The choice of the sequence of steps is up to the preference of the investigator and is not decisive for the result. It should be taken into account, that the prior art is not required to disclose identically the process as disclosed in the so-called examples, but rather should correspond to the teaching of the claims. Claim 1, however, does not recite any process step, whereas claim 2 requires

(1) a prior (ultra) filtration with molecular weight cut-off at 10 kD
(2) lectin affinity chromatography
(3) gel filtration or ion exchange chromatography.

D3, once again, applies

(1) ultra filtration using Amicon Y-10 i.e., 10 kD molecular weight cut-off
(2) gel filtration (Sephacryl S200) and
(3) lectin affinity chromatography (Concanavalin A).

Thus, the only difference between the method according to **D3** and that required by claim 2 is the reversal of steps 2 and 3. This it not sufficient to establish an inventive step.

5.1.3 With respect to the argument that despite similar biological activities proteins can vary with respect to their structure, the proprietor's submission is correct. They <u>can</u> vary, but they do not have to, and a person skilled in the art would not necessarily expect them to do. In any case, if a person skilled in the art would have to decide between the purification protocol for a protein known to have comparable effects to those of another protein, he certainly would try to modify the already available protocol in order to adapt same to the new purpose.

5.1.4 The suitable source for obtaining TNF-inhibitor has already been discussed in 5.1.1 above. In view of the available knowledge at that time, urine was certainly the source of choice.

Summarising the above, the subject-matter of claims 1 and 2 and dependent claims is already obvious over the teaching of **D3**. It is even more when **D3** is combined with **D7** or **D2**.

5.1.5 The subject-matter of claims 1 to 10 lacks also an inventive step over **D7**, **D8**, **D9**, **D11** and **D12**. As discussed in the opposition brief, all of the aforementioned documents disclose the existence of TNF receptor cross linked to TNF-α. **D7** in addition discloses the release of TNF-α from the cross linked molecule SDS gel, thus leaving behind free TNF receptor. We further submit **DA47** (Brockhaus et al, PNAS, 87: 3127-3131, 1990), cited as an expert's opinion and confirming that TNF binding protein separated on SDS-PAGE is still

active, i.e., is still capable of binding TNF-α. See **DA47,** paragraph bridging page 3129 and 3130, and the description of Fig. 5.

Considering this information, it is apparent that it is not only obvious to try to isolate a protein according to claim 1 or 2, but there is also a considerable expectation of success.

5.2 Lack of an inventive step of claims 3 to 10 over D16

As already discussed above, **D16** is considered to fully anticipate the subject-matter of claim 3. If, however, the Opposition Division should not share our view with respect to **D16**, it is submitted that the subject-matter of claim 3 is at least not inventive over this document. The same applies to claims 4 to 10.

D16 undoubtedly discloses the same protein as the opposed patent. The protein was purified by a series of steps including ultrafiltration, ammonium sulphate precipitation and Sephacryl S200 gel filtration or chromatofocussing. Certainly, there is no inventive step in further purifying a protein which is already purified to the extent that it elutes "as a single peak". In addition the invitation to apply further purification steps is already given in **D16** - see page 1515, second sentence of section "Discussion".

Moreover, the proprietor recognized that the information regarding pI (which was obtainable from **D16**) was a sufficient parameter of the factor that could be used for further purification. Specifically, we refer to **DA48** cited as an expert's opinion. On page 37 of his thesis, Dr. Engelmann states the following:

> "Size exclusion chromatography resulted in poor resolution between the proteins exerting TNF inhibitory activities, peaking in the range of 50 to 70 kD, and the bulk of urinary proteins. Some enrichment of the protein(s) of interest was obtained in preparative isoelectric focusing. Consistent with prior observations the majority of urinary proteins was found in the acidic pII range from 3.0 to 4.5 while the proteins protecting against TNF cytotoxicity were found at a pII range of 5.7 to 6.3 (Fig. 5). Fractionation by isoelectric focusing as an initial step of enrichment seemed impractical since only limited amounts of proteins could be applied at a time. However the pattern of isoelectric points revealed by isoelectric focusing indicated that it should be possible to effectively enrich the protective protein(s) by their charge properties".

The opposed patent teaches purification by the steps of microfiltration (size exclusion), ion exchange chromatographies (to separate proteins by charge), gel filtration (size exclusion) and reversed phase HPLC (to separate proteins by hydrophobicity). Interestingly, this methodology generally tracks the purification scheme of **DA49**. Specifically, in that reference the authors partially purified an IL-1 inhibitor by subjecting urine to ultrafiltration (size exclusion), ammonium sulfate fractionation followed by ion exchange chromatography (to separate proteins by charge), hydroxyapatite chromatography (to separate proteins partially by hydrophobicity) and concentrated by gel filtration (size exclusion).

It is respectfully submitted that no inventive activity can be recognised in improving the purity of an already purified protein by already known methods (one of which is even mentioned in **D16**). In this connection, it is referred to **T877/90** (Hooper Trading/T-cell growth factor) where the Board of Appeal had to consider the inventive step of a T-cell growth factor being serum and mitogen free over a T-cell growth factor containing serum and mitogen contaminants. The Board held (point 3.1.5 of the decision):

> Being equipped with the information that neither mitogen nor serum are desired in a pharmaceutical acceptable end product it would have been the next obvious and evident step for the skilled person within the meaning of an obvious *desideratum* to further develop the substance whose activity was determined in document (17) to make the product completely mitogen and serum free.

The claim was therefore considered not to involve an inventive step.

The same considerations as outlined above for a serum and mitogen free T-cell growth factor should apply to the present case. For compounds intended for pharmaceutical purposes as the present one any kind of contaminant is undesired, whether their identity is known or not.

If the proprietor in fact assumes that an inventive step is involved in the subject-matter of claim 3 he ought to explain why this should be the case. In the absence of any particular difficulty involved in the further purification and considering the fact that the additional steps chosen by the proprietor are just common steps routinely used for the purification of a variety of proteins, no

inventive step can be acknowledged. It should also be noted that to the best of our knowledge there is no precedent of a case, where the mere purification has been considered inventive.

5.3 Inventive Step of claims 11 and 12

The proprietor denies that **D16** refers to **D14** for purification purposes. He further alleges that the method of claim 11 is "completely different" from the method according to **D14** (point 7.5.2 of proprietor's letter). Both statements are incorrect.

5.3.1 **D16** on page 1511 explicitly refers to **D14** for the purpose of concentration and precipitation. See "Materials and methods", first paragraph.

5.3.2 The process according to **D14** is not completely different from that of claim 11. There is only one step missing, i.e., the step of reversed phase HPLC. The fact that **D14** applies additional steps is irrelevant, since claims 11 and 12 do not exclude such additional steps. Further, the process according to claims 11 and 12 does not require, as alleged by the proprietor, that "several ion exchange chromatographies" are conducted. Rather, this is a feature of dependent claim 13.

Consequently, **D14** teaches the method according to claims 11 and 12 with the exception of reversed phase HPLC. As stated above, purifying urine-derived proteins (e.g., IL-1 inhibitor) was known. Reversed phase HPLC was a well known method even before the European filing date, as shown by **D15**. Upon supplementing the method of **D14** with the teaching obtainable, e.g., from **D15**, a method comprising each and any process step according to claim 11 is obtained. However, in the opponent's view it is not even necessary to cite a document disclosing reversed phase HPLC, since use thereof was a method which was sooner or later applied by any person attempting to isolate a protein. Therefore, it is submitted, that **D16** in combination with **D14** and the average skilled person's knowledge directly and straight forward leads to the method according to claims 11 and 12. Therefore, neither of these claims does involve an inventive step.

6. Requests

In view of the above our requests as submitted with letter dated August 30, 1995 prove to be justified. Therefore, the following requests are maintained:

1. Revocation of the patent in full
2. Scheduling of oral proceedings in the event that the Opposition Division is not inclined to follow the first request.

7. Participants in oral proceedings

In addition to the undersigned, the following persons, all of Amgen Inc., are likely to attend oral proceedings:

1. Mr. Steven Odre, Vice President Intellectual Property
2. Mr. Thomas Zindrick, Corporate Counsel
3. Dr. Carl Edwards III, sTNF-RI Project Leader
4. Dr. Tadahiko Kohno, Senior Scientist
5. Mr. Michael Brewer, Associate Scientist
6. Mr. Greg Brown, Associate Scientist.

All of these persons have a detailed knowledge on legal and/or technical questions connected with the subject-matter of the opposed patent. Therefore, it is requested that these persons are permitted to provide comments and explanations on relevant issues e.g., novelty, inventive step or enablement, once this should appear to be appropriate, during oral proceedings.

(Heike Vogelsang-Wenke)

Encl: Four copies of this letter
Four copies of **DA24** to **DA54**
Annex 1 (List of newly cited references)*
Annex 2 (Table of contents) (to follow)
Annex 3 (Crosslinking experiment)*
Annex 4 (Homology Search)* (* 4-fold)